Currently Pending Claims

This listing of claims will replace all prior versions and listings of claims in the application:

1. (**Currently Amended**) A method for generating a secondary library of protein sequences **with at least one desired characteristic relative to** [[of]] a target protein comprising:

a) inputting the coordinates of said target protein into a computer;

b) **selecting a plurality of positions from said target protein based upon said at least one desired characteristic to generate a set of primary variant positions;**

c) applying a forcefield calculation to ~~**utilizing**~~ said coordinates**[[,]]and said set of primary variant positions** ~~**and a forcefield calculation**~~ to generate a primary library of primary **variant** protein sequences comprising a plurality of primary variant amino acid residues at **each of said** primary variant positions;

[[c]]d) generating a probability distribution of amino acid residues **[[in a]] at each of said** plurality of **primary** variant positions **in said primary variant protein sequences** ~~**from said force field calculation**~~;

[[d]]e) combining **at each of said primary variant positions** a plurality of said amino acid residues from said probability distribution **into a plurality of said primary variant positions in said target protein sequence** to generate a secondary library of secondary **variant protein** sequences; wherein at least one of said secondary **variant** protein sequences is different from said primary **variant** protein sequences; and

[[e]]f) synthesizing **and screening for said at least one desired characteristic** a plurality of said secondary **variant** protein sequences.

2. (**Currently Amended**) A method for generating a secondary library of protein sequences **with at least one desired characteristic relative to [[of]]** a target protein comprising:

a) inputting the coordinates of said target protein into a computer;

b) **selecting a plurality of positions from said target protein based upon said at least one desired characteristic to generate a set of primary variant positions;**

c) applying a forcefield calculation to ~~**utilizing**~~ said coordinates**[[,]] and said set of primary variant positions** ~~**and a forcefield calculation**~~ to generate a primary library of primary **variant** protein sequences comprising a plurality of primary variant amino acid residues at **each of said** primary variant positions;

[[c]]d) generating a probability distribution of amino acid residues **[[in a]] at each of said** plurality of **primary** variant positions **in said primary variant protein sequences** ~~**from said force field calculation**~~;

[[d]]e) generating a set of oligonucleotide probes each encoding at least one of said primary variant amino acid residues;

[[e]]f) using said probes in a polymerase chain reaction (PCR) to generate a plurality of oligonucleotide sequences, each encoding a plurality of secondary variant protein sequences, wherein at least one of said secondary variant protein sequences is different from said primary protein sequences; and,

[[f]]g) producing **and screening for said at least one desired characteristic a plurality of** said secondary variant protein sequences in host cells transformed with said oligonucleotide sequences.

3. (Previously Presented) A method according to claim 2 wherein said PCR is multiple PCR and wherein said probes are pooled.

4. (Previously Presented) A method according to claim 3 wherein said probes are added in equimolar amounts.

5. (Previously Presented) A method according to claim 3 wherein said probes are combined in amounts that correspond to the probability of said variant amino acid residues in said probability distribution table.

6. (Cancelled)

7. (Previously Presented) A method according to claim 1 wherein said target protein is an enzyme.

8. (Previously Presented) A method according to claim 1 wherein said target protein is a therapeutic protein.

9. (Previously Presented) A method according to claim 1 wherein the coordinates of a region surrounding a binding site to a receptor is input into a computer.

10. (Previously Presented) A method according to claim 1 wherein said primary variant positions comprise a region surrounding a binding site.

11. (Previously Presented) A method according to claim 8 wherein said primary variant positions comprise a region surrounding a binding site to a receptor.

12. (Previously Presented) A method according to claim 7 wherein said primary variant positions comprise a region surrounding the active site of said enzyme.

13. (Previously Presented) A method according to claim 7 wherein said primary variant positions comprise a region surrounding the catalytic residues of said enzyme.

14. (**Currently Amended**) A method for generating a secondary library of protein sequences **with at least one desired characteristic relative to** [[of]] a target protein comprising:

(a) generating a primary library comprising:

(i) inputting the coordinates of a target protein ~~with variable residue positions~~;

(ii) **selecting a plurality of positions from said target protein based upon said at least one desired characteristic to generate a set of primary variant positions;**

(iii) establishing a group of potential amino acids for each of said ~~variable residue~~ **primary variant** positions, wherein the group of potential amino acids for at least one of said ~~variable residue~~ **primary variant** position comprises at least two different amino acid side chains; and

[[(iii)]](iv) analyzing the interaction of each of said **potential** amino acids with **a** plurality of said amino acids at a plurality of ~~variable residue~~ **primary variant** positions and all or part of the

remainder of said protein to generate a primary library of primary **protein** sequences;

(b) generating a probability distribution of amino acid residues from said primary library **[[In]]** **at each of** a plurality of **primary** variant positions from said primary **protein** sequences;

(c) combining **at each of said primary variant positions** a plurality of said amino acid residues from said probability distribution **into a plurality of said primary variant positions in said target protein sequence** to generate a secondary library of secondary **variant protein** sequences ~~**comprising secondary variants**~~; wherein at least one of said secondary variant**[[s]]** **protein sequences** is different from said primary variant**[[s]]** **protein sequences**; and

(d) synthesizing **and screening for said at least one desired characteristic** a plurality of said secondary **variant** protein sequences;

wherein at least one of said analyzing, generating or combining steps comprises using a force field calculation.

15. (**Currently Amended**) A method for generating a secondary library of protein sequences **with at least one desired characteristic relative to** [[**of**]] a target protein comprising:

(a) generating a primary library comprising:

(i) inputting the coordinates of a target protein ~~**with variable residue positions**~~;

(ii) **selecting a plurality of positions from said target protein based upon said at least one desired characteristic to generate a set of variable residue positions;**

(iii) establishing a group of potential rotamers for each of said variable residue positions, wherein the group of potential rotamers for at least one of said variable residue position has a rotamer selected from each of at least two different amino acid side chains; and

[[(iii)]](iv) analyzing the interaction of each of said rotamers with plurality of said rotamers at a plurality of variable residue positions and all or part of the remainder of said protein to generate a primary library of primary sequences;

(b) generating a probability distribution of amino acid residues from said primary library in a plurality of variant positions from said primary sequences;

(c) combining a plurality of said amino acid residues **at each of said variable residue positions** from said probability distribution to generate a secondary library of secondary sequences comprising secondary variants; wherein at least one of said secondary variants is different from said primary variants; and,

(d) synthesizing **and screening for said at least one desired characteristic** a plurality of said secondary protein sequences,

wherein at least one of said analyzing, generating or combining steps comprises using a force field calculation.

16. (Previously Presented) A method according to claim 14, wherein said force field calculation is Self-Consistent Mean Field (SCMF).

17. **(Currently Amended)** A method for generating a secondary library of protein variants **with at least one desired characteristic relative to** [[of]] a target protein comprising:

(a) generating a primary library comprising:

(i) inputting the coordinates of a target protein ~~with variable residue positions~~;

(ii) **selecting a plurality of positions from said target protein based upon said at least one desired characteristic to generate a set of variable residue positions;**

(iii) establishing a group of potential rotamers for each of said variable residue positions, wherein the group of potential rotamers for at least one of said variable residue position has a rotamer selected from each of at least two different amino acid side chains; and

[[(iii)]](iv) analyzing the interaction of each of said rotamers with plurality of said rotamers at a plurality of variable residue positions and all or part of the remainder of said protein to generate a primary library of primary **protein** sequences optimized for at least one scoring function;

(b) generating a probability distribution of amino acid residues from said primary library in a plurality of variant positions from said primary **protein** sequences;

(c) combining a plurality of said amino acid residues from said probability distribution to generate a secondary library of secondary **protein** sequences comprising secondary variants; wherein at least one of said secondary variants is different from said primary variants; and,

(d) synthesizing **and screening for said at least one desired characteristic** a plurality of said secondary protein sequences,

wherein at least one of said analyzing, generating or combining steps comprises using a force field calculation.

18. (Previously Presented) A method according to claim 17, wherein said scoring function is selected from the group consisting of a van der Waals potential scoring function, a hydrogen bond potential scoring function, an atomic solvation scoring function, an electrostatic scoring function and a secondary structure propensity scoring function.

19. (Previously Presented) A method according to claim 14, 15, or 17, wherein said analyzing step utilizes a force field calculation.

20. (Previously Presented) A method according to claim 14, 15, or 17, wherein said generating step (B) utilizes a force field calculation.

21. (Previously Presented) A method according to claim 14, 15, or 17, wherein said combining step utilizes a force field calculation.

22. **(Currently Amended)** A method for generating a secondary library of protein sequences **with at least one desired characteristic relative to** [[of]] a target protein comprising:

a) inputting the coordinates of said target protein into a computer;

b) specifying a list of at least two primary variant positions **based upon said at least one desired characteristic**;

c) **applying a forcefield calculation to** ~~**utilizing**~~ said coordinates**[[,]]and said primary variant positions** ~~**and a forcefield calculation**~~ to generate a primary library comprising a plurality of primary variant amino acid residues at **each of** said primary variant positions;

d) generating a probability distribution of amino acid residues **[[in a]] at each of said** plurality of **primary** variant positions **in said primary variant protein sequences** ~~**from a force field calculation**~~;

e) combining **at each of said primary variant positions** a plurality of said amino acid residues from said probability distribution **into a plurality of said primary variant positions in said target protein sequence** to generate a secondary library of secondary **variant protein** sequences; wherein at least one of said secondary **variant** protein sequences is different from said primary **variant** protein sequences; and

f) synthesizing **and screening for said at least one desired characteristic** a plurality of said secondary protein sequences.

23. (Previously Presented) A method according to claim 22 wherein said primary library is generated using a monte carlo search.

24. (Previously Presented) A method according to claim 22 wherein said primary library is generated using a genetic algorithm.

25. (Previously Presented) A method according to claim 22 wherein said probability distribution table is derived from frequencies of occurrence in a primary variant library.

26. (Previously Presented) A method according to claim 1 wherein combining said plurality of said amino acid residues from said probability distribution comprises a calculation in said computer.